ISOELECTRIC FOCUSING OF BOAR SPERMATOZOA

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Summary. The isoelectric points of washed spermatozoa from intact boars and from boars after removal of the seminal vesicles were determined using isoelectric focusing on natural pH gradients. Normal boar spermatozoa focused at a higher pH than spermatozoa from boars without seminal vesicles. The isoelectric point of the latter was increased to a value approaching normal by preincubation in normal seminal plasma. This indicates that seminal plasma alters the membrane surface charge of boar spermatozoa on ejaculation.

The surface properties of cells are mainly attributable to the distribution of ionizable chemical groups on the cell membrane. Electrophoretic techniques have long been used to determine the net charge of spermatozoa (Mudd & Mudd, 1929). Nevo, Michaeli & Schindler (1961) demonstrated that live bull and rabbit spermatozoa have a net negative charge although the polarization of the cells indicated that the distribution of charge was uneven. As spermatozoa pass through the epididymides their net surface charge increases (Bedford, 1963). The distribution of the surface charge on the spermatozoa of a variety of species has been extensively studied with the use of positively charged particles of colloidal iron hydroxide (Yanagimachi, Noda, Fujimoto & Nicolson, 1972). By adapting existing techniques of isoelectric focusing, it is possible to determine the isoelectric point of intact cells (Sherbet, Lakshmi & Rao, 1972; Sherbet & Lakshmi, 1973). To assess the binding occurring between the seminal plasma proteins and the spermatozoa, we used similar methods for measurements of the isoelectric point of intact washed spermatozoa of two normal boars and of three boars from which the seminal vesicles had been removed (Davies, Hall, Hibbitt & Moore, 1975).

A small perspex column (Text-fig. 1) was manufactured in the Institute's workshop. With this column, a pH gradient could be rapidly generated on which spermatozoa could be placed. The pH and density gradient was prepared by the following method. Ampholines (pH 3.5 to 10: LKB Ltd, Bromma, Sweden) were added to equal volumes of 2% and 15% (w/v) ficoll solutions to give a final ampholine concentration of 1% (v/v). The solutions, mixed in a gradient maker, formed a 2 to 15% ficoll density gradient in the column which supported the pH gradient produced by passing a constant current of 1 mA for 16 hr. The electrode solutions were 1 M-NaOH (cathode) and 1 M-H₃PO₄ (anode) made up to the appropriate density with ficoll. Spermatozoa from intact and vesiculectomized boars were washed in 0.9% NaCl, centrifuged at

TEXT-FIG. 1. Small perspex column used for focusing spermatozoa. Elution of the gradient was by way of a 'flow-through' pH electrode. A, anode; C, cathode; E, electrofocusing compartment (20 ml); F, flow-through pH electrode; R, rubber septum for sample injection tap.

700 *g* for 10 min and resuspended in 0.3 ml of the pH/ficoll gradient removed from the column with a 1-ml syringe through the rubber septum. The spermatozoa (5×10^6) were then injected onto the column through the septum and allowed to focus for 2 hr at 2 mA. Determinations were made of the isoelectric points of spermatozoa from three semen collections for each boar. The gradient containing the focused spermatozoa was run from the column through a 0.5-ml 'flow-through' pH electrode connected to a pH meter (Pye Unicam, Cambridge) and collected in 0.5-ml fractions. The spermatozoa were detected by measuring the absorption of the fractions at 720 nm (Rank Uvichem 2, Camden, London) and by making sperm counts (Neubauer haemocytometer).

Spermatozoa from intact boars focused on the gradient at $\text{pH } 6.5 \pm 0.35$ while the spermatozoa from boars without seminal vesicles focused at $\text{pH } 4.5 \pm 0.25$ (Text-fig. 2). When spermatozoa from vesiculectomized boars were incubated in normal seminal plasma for 10 min and then washed in saline, before being injected onto the column, the isoelectric point increased to $\text{pH } 5.8 \pm 0.3$. After focusing, the cells were intact but immotile. In two experiments, a small band of cells was observed at the acidic end of the gradient to one side of the main band. This was later identified as containing cellular fragments. Reversal of the current and electrode solutions did not affect the results,

TEXT-FIG. 2. Elution profiles for spermatozoa from intact and vesiclectomized boars with corresponding pH density gradients. $\blacktriangle$, Spermatozoa from intact boars, and gradient ($\bullet$); $\triangle$, spermatozoa from vesiclectomized boars, and gradient ($\circ$).

demonstrating that the focusing was due to charge and not to specific gravity.

In the boar, most of the seminal plasma proteins are cationic and have isoelectric points between pH 8.2 and 9.4 (Bournsnel, Johnson & Zamora, 1962; Bournsnel & Briggs, 1969). These proteins are secreted from the seminal vesicles (Bournsnel & Coombs, 1966), hence, removal of the glands depletes the seminal plasma of basic proteins and reduces its total protein content to less than 10% of that of intact boars (Davies *et al.*, 1975). The change in the isoelectric point of boar spermatozoa after vesiclectomy indicates that seminal vesicle proteins alter the surface charge of spermatozoa on ejaculation. As seminal vesicle basic proteins have been shown to bind to the sperm membrane on ejaculation (H. D. M. Moore and K. G. Hibbitt, unpublished), it is probable that the rise in the isoelectric point of spermatozoa from vesiclectomized boars after incubation with seminal plasma was due to the binding of these proteins to the spermatozoa.

This technique offers a means for monitoring the surface charge of spermatozoa during the maturation and fertilization processes. With specific chemical treatments such as incubation with neuraminidase and methylation, changes in the isoelectric point of spermatozoa may reflect the type of membrane surface groups present. The small column used in this study allows determinations to be made with relatively few cells.

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